ON THE DEVELOPMENT OF PROTEOLYTIC FERMENTS IN THE BLOOD DURING PNEUMONIA.*

GEORGE F. DICK.

(From the Memorial Institute for Infectious Diseases, Chicago.)

In the pneumonic crisis we have an extremely rapid recovery which, could it be brought about at will, would represent an ideal therapeutic result. An understanding of the processes responsible for this change is therefore especially desirable.

With the hope of finding differences in the blood of pneumonic patients before and after crisis, which might be held accountable for its occurrence, the various antibodies commonly developed in infectious diseases have been extensively studied. Recently the opsonic and phagocytic power of the blood has received the greatest amount of attention (Wolf, MacDonald, DeMarchis). Rosenow[1] also studied the opsonic power of the serum of pneumonic cases, using non-virulent strains, and found a constant increase in opsonic content of the blood at the time of crisis. With virulent organisms, however, phagocytosis did not occur. Later, in a study of the lung exudates obtained by aspiration from the lungs of patients,[2] he found that phagocytosis occurred, but that whenever it was found the organisms outside the leukocytes showed evidence of disintegration. Rosenow concludes that while the opsonins take part in the defense of the body against pneumococci, their rôle in the process of the crisis is a secondary one. Strouse[3] reaches a similar conclusion. Eggers[4] obtained by the plate method evidences of an increased antipneumococcal power of the blood at or shortly after the crisis, and attributes this action, at least in part, to opsonins. Neufeld and Händel[5] claim that the serum of postcritical pneumonia patients protects mice against fatal doses of pneumococci. Neufeld and Händel[6] and Kyes[7] have produced serum by immunization which protects mice against pneumococcus infections. Moro[8] observed an increase in the complement content of the blood after crisis.

The importance of the splitting of bacterial proteins in infections has been established by Vaughan,[9] Friedberger and his associates,[10] Pfeiffer and Mita,[11] Rosenow,[12] and Neufeld and Dold.[13] These investigators have shown that increased toxicity occurs with proteolysis up to a certain point beyond which further splitting decreases the toxicity of the protein. The production of protein-splitting ferments by the injection of protein antigens has been demonstrated by Abderhalden and his

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[1] *Jour. Infect. Dis.*, 1906, 3, p. 683.

[2] *Ibid.*, 1911, 8, p. 500.

[3] *Jour. Exper. Med.*, 1911, 14, p. 109.

[4] *Jour. Infect. Dis.*, 1912, 10, p. 48.

[5] *Arb. a.d.k. Gsndhtamte.*, 1910, 34, p. 166.

[6] *Loc. cit.*

[7] *Jour. Am. M. Ass.*, 1911, 56, p. 1878.

[8] *Ueber das Verhalten hemolytischer Serumstoffe beim gesunden und kranken Kind*, Wiesbaden, 1908.

[9] *Jour. Infect. Dis.*, 1907, 4, p. 476.

[10] *Deutsche med. Wchnschr.*, 1911, 37, p. 377.

[11] *Ztschr. f. Immunitätsf.*, 1911, 6, p. 18.

[12] *Jour. Infect. Dis.*, 1912, 10, p. 113.

[13] *Berl. klin. Wchnschr.*, 1911, 48, p. 1069.

associates.[1] I have shown[2] that these proteolytic ferments may be specific and capable of inactivation and reactivation.

The following experiments were attempted in order to find out if such ferments were demonstrable in pneumonia or not. On account of the importance of complement in anaphylaxis[3] the complement estimations of Moro were repeated.

An extract of pneumococci was prepared by growing virulent pneumococci in ascites or serum broth in large quantities and centrifugating the cultures. The broth was then pipetted off and the bacterial sediment heated to 60° C. for one-half hour in order to destroy the autolytic ferments. The heated bacteria were then dried and ground thoroughly with sand and extracted with salt solution for 24 hours in the ice-box. The salt solution used was half normal so that the strength of the extract could be varied by adding salt solution or by evaporation. The extract was then cleared by centrifugation, filtration through paper pulp, and finally when necessary through porcelain filters. The extract was now diluted so as to give a levorotation of about 30′–45′ with a 10 c.c. tube, and then sterilized by heating to 60° C. for one hour.

Blood was obtained from the arm veins of pneumonia patients at various periods of the disease, before and after crisis, and the serum removed by centrifugation. A mixture of 1 c.c. of serum and 10 c.c. of pneumococcus extract was made and the optical activity estimated as quickly as possible after mixing thoroughly. The mixture was then incubated for four hours and a second reading made. The results are given in tabular form.

The complement was estimated by the following method: Tubes were arranged with 0.1, 0.2, 0.3, 0.4, and 0.5 c.c. of a dilution of 1–100 of the serum to be tested. A corresponding set made with normal serum served as a standard. To each tube was added 10 times the minimum amount of amboceptor required to produce complete hemolysis with 0.1 c.c. of a 1–10 dilution of normal serum; 0.2 c.c. of a 5 per cent suspension of sheep's blood were then added and the mixture made up to 1 c.c. Controls were made without amboceptor to guard against the estimation of the

[1] *Ztschr. f. physiol. Chem.*, 1910, 64, pp. 100, 423, 425, 427.

[2] *Jour. Infect. Dis.*, 1911, 9, p. 282.

[3] Friedberger and Hartoch, *Ztschr. f. Immunitätsf.*, 1909, 3, p. 581.

total hemolytic power of the serum rather than the complement concentration. The mixtures were incubated for two hours and then placed on ice for 18–24 hours and examined. The figures in the table refer to the number of 0.1 c.c. parts in the test serum and the standard serum giving the same amount of color to the fluid over the sedimented corpuscles; for instance, 3 would mean that 0.1 c.c. of the test serum equaled in complement action 0.3 c.c. of normal serum, $\frac{1}{3}$ would mean that 0.3 c.c. of test serum equaled 1 c.c. of normal serum.

Case	Description of Case	Optical Rotation of Mixture in Minutes		Difference in Rotation	Complement
		Before Incubation	After Incubation		
1	Third day of disease	40′	40′	0′	$\frac{1}{3}$
2	Fourth day of disease	48′	48′ ?	0′	1
3	Sixth day of disease	37′	37′	0′	$\frac{1}{3}$
4	Eighth day of disease beginning lysis	47′	46′	1′	$\frac{1}{2}$
5	Normal serum	41′	40′	1′	1
6	Normal serum	37′	36′	1′	1
7	Two days after crisis	39′	31′	8′	3
8	Three days after crisis	46′	40′	6′	1
9	Four days after crisis	42′	35′	7′	2
10	Six days after crisis	36′	35′	1′	2
11	Ten days after crisis	43′	34′	9′	$1\frac{1}{2}$
12	Ten days after crisis	40′	34′	6′	2
	Extract alone, control	31′	30′	1′	
	Serum from case 9 alone	12′	12′	0′	

The table shows a distinct decrease in the optical activity of the mixtures made with the serum after crisis and none with the normal serum or the precritical pneumonic serum. Case 10 represents an exception to this as well as one other case (14). In neither case was there anything peculiar in the clinical course to explain the difference. Inasmuch as blood cultures were not made, however, it is possible that these were not pneumococcus infections, although they represented clinically typical cases. According to Abderhalden, this decreased optical activity would indicate that digestion of the extract had taken place. In order to decide whether the ferments in postcritical serum were specific or whether they might be connected with the process of the resolution and removal of the pneumonic exudate, serum from postcritical pneumonics was compared as to its action on pneumococcus extracts, colon bacillus extracts, and typhoid bacillus extracts. The results are as follows:

Case 13, Comp. = 1	Pneumococcus Extract	Colon Bacillus Extract
Before incubation	54′	48′
After incubation	50′	47′
Difference in rotation	4′	1′

Case 14, Comp. = 1	Pneumococcus Extract	Colon Bacillus Extract
Before incubation	47′	40′
After incubation	45′	40′
Difference in rotation	2′	0′

Case 15, Comp. = 2	Pneumococcus Extract	Typhoid Bacillus Extract
Before incubation	48′	49′
After incubation	44′	50′
Difference in rotation	4′	1′

These results would indicate that the proteolytic action was specific for pneumococci.

It will be noted that except in cases 8 and 13 the hemolytic complement content of the blood is higher in those cases having a proteolytic power. In order to further investigate this relationship the serum of case 9 (after crisis) was heated to 60° C. for one-half hour and a mixture made of 0.5 c.c. of heated serum, 1 c.c. of normal serum, and 10 c.c. of pneumococcus extract. The polariscopic reading was 48′, and after incubation 44′. Two serums having different proteolytic power (cases 1 and 7) were heated to 60° C. for one-half hour and then mixtures made of 1 c.c. of heated serum, 0.5 c.c. of guinea-pig serum, and 10 c.c. of pneumococcus extract. The results were as follows:

Case 1. Before incubation 41′ After incubation 40′
Case 7. " " 41′ " " 38′

It will be seen that although the guinea-pig serum contains about eight times the hemolytic complement content of normal human serum and that although the content complement in guinea-pig complement was made equal in the serums of cases 1 (before crisis) and 7 (after crisis), there is still a difference in the proteolytic action. The significance of the difference in hemolytic comple-

ment content of the blood before and after crisis requires further investigation.

CONCLUSIONS.

Proteolytic ferments develop in the blood during pneumonia about the time of crisis. These ferments seem to have a special action upon pneumococcus protein and may take part in the mechanism of the crisis.